

## HKSTP IACUC

### 14 – Breeding of Laboratory Animals Guidelines

#### *Version History*

Version	Effective Date
1	28/02/2023

#### 1. Purpose

The purpose of the guidelines is to address the health and well-being of animals by ensuring safe population densities. Animal overcrowding can contribute to significant animal welfare issues and therefore may violate policies on the humane care of animals if not expediently addressed.

Breeding schemes and strategies shall be managed and maintained properly to produce sufficient animals for experiments and minimize number of unwanted animals (presentation of overbreeding). Overbreeding is a major animal welfare issue, it involves breeding an animal more than its body can safely handle resulting in detrimental health effects to the mother and her pups as well as the overpopulation and subsequent euthanasia of many unwanted animals. Since animal welfare and rationale for animal numbers is part of HKSTP IACUC's ethical review, justification for the number of breeding animal and the number of offspring used for research studies are required to be documented and provided to HKSTP IACUC for review and monitoring on demand basis.

#### 2. General Requirements

2.1 The following information shall be available upon HKSTP IACUC's request:

- i. Predicted numbers of animals generated based on breeding strategy,
- ii. Predicted numbers of animals that can be used for experiments based on genotypes needed (keeping in mind whether predicted genotypes will be generated according to Mendelian genetics).
- iii. Breeding scheme, weaning age, duration that breeders are used, method for obtaining tissue samples for genotyping, method for identifying pups (ear punch, toe clips, animal ID tags, or tattoo)
- iv. Any other pertinent information related to breeding procedures.

2.2 For pregnant or parturient animals, at least 10% greater cage floor area than set out in relevant standards normal for the species should be provided, with an environment and conditions suitable for giving birth.

### 3. Types of Breeding Schemes [Rodent Specific]

#### 3.1 Pairs (one male, one female)

Breeding cages may be set up on a continuous basis, leaving the male with the female(s) after pups are born. Simultaneous presence of two or more litters in a single cage is not recommended, unless scientifically justified. Weaning schedules should be strictly managed to ensure that cages do not become overcrowded.

For continuous breeding, pairs breeding scheme is recommended under the following factors:

- i. The strain produces large numbers of offspring ( $\geq 10$  pups/litter)
- ii. Weaning needs to be delayed beyond 21-23 days due to the small size of offspring and their inability to thrive (as occurs for some inbred strains).

#### 3.2 Trios (one male, two females)

Breeding cages may be set up on a continuous basis, leaving the male with the female(s) after pups are born. Simultaneous presence of two or more litters in a single cage is not recommended, unless scientifically justified. Weaning schedules should be strictly managed to ensure that cages do not become overcrowded. Policies and management techniques are required to allow pups to develop to weaning age without detrimental effects for the mother or litters.

For mice strains that regularly produces large litters ( $\geq 10$  pups), the litters should be weaned between 21-23 days. For inbred strains that produce large litters ( $\geq 10$  pups) and require 28 days for proper weaning, scientific justifications for the need for trio breeding with delayed weaning is required.

#### 3.3 Quintet/ Quartet (one male, three/four females)

Each visibly pregnant female should be moved to separated individual cage to prevent housing multiple pre-weaning litters with more than 3 adults. Separation of pregnant females ensure well-being of newborn animals, as well as to provide adequate data regarding birth and weaning dates, cages should be adequately labeled by the investigator with the date of birth of each litter.

- i. Enrichment and accessible food and water must be provided for every animal cage (including newly weaned pups) unless scientifically justified. Additional forms of enrichment are required for singly housed animals.
- ii. Breeding males should be singly housed after being separated due to their aggressive nature after breeding. Breeding males may cause grave physical harm or death to a male cage mate if cohoused.

Note: Overcrowding should be avoided. Pregnant females should be separated into individual maternity cages for delivery of litters. The IACUC recommends separation of pregnant females up to one week before expected delivery. Maternity cages should be closely observed for delivery of pups and the date of birth recorded on cage cards. Simultaneous presence of two or more litters in a single cage is not recommended, unless scientifically justified.

#### 4. Weaning [Rodent Specific]

- 4.1 Research Staff shall be responsible for cage card documentation and for separating and weaning mice. Litters should be weaned according to the procedures defined in the protocol.
- 4.2 Male and female pups should be separated at weaning unless they are being set up as new breeding cages.
- 4.3 All litters should be weaned between 21 – 23 days of age. Pups should be separated by sex into individual male or female cages, keeping in mind of animal housing density. Exceptions to weaning age (beyond 23 days) should be scientifically justified.
- 4.4 Weaned pups should have a minimum of 2 mice per cage. In the case of a lone female, she may stay with the mother. In the case of a lone male, he should be paired in the same cage with a similarly aged litter of the same strain. If not possible, extra enrichment should be provided.
- 4.5 Litters of 21 days or younger should NOT be placed in a cage with a lactating female that also has a newborn litter.
- 4.6 In the event that litters cannot be weaned according to guidelines, appropriate cage labeling the cage with the expected weaning date shall be recorded and provided to HKSTP IACUC on demand basis.

#### 5. Cage Identification

5.1 All breeding cages shall be marked. The following information shall be included:

- i. Name of PI
- ii. Number of females in cage
- iii. Set up date of breeding cage
- iv. Date of birth of litters
- v. Weaning date

#### 6. Recommendations [Rodent Specific]

- 6.1 Post Weaning. Adult females may be recycled into a breeding cage with a male for production of another litter.
- 6.2 Additional Monitoring. A specific genetic modification that routinely results in a longer period before weaning should be noted in the IACUC protocol. Any changes or updates to approved breeding procedures must first be submitted as an amendment for review and approval by the IACUC.
- 6.3 Contact Veterinary Staff. Any mouse pups weighing less than 10 grams at 23 days should be reported to veterinary staff for evaluation. If any other animal care concerns are noted, please contact ULAR for additional guidance and assistance.

6.4 Retirement of Breeding Rodents. Fertility of rodents is highest during the first 6 months of their lives. After a year of age, fertility is very low. Therefore, the IACUC recommends retiring breeders between 8 to 12 months of age. Individual genetic lines will vary, and investigators should use their best judgment and experience to decide when to replace breeders.